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(FILE 'HOME' ENTERED AT 14:39:24 ON 24 MAY 2006)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 14:39:35 ON 24 MAY 2006

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13	FILE CONFSCI
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1	FILE VETU
61	FILE WPIDS
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L1 QUE IDURONIDASE

FILE 'BIOSIS, EMBASE, MEDLINE, SCISEARCH, CAPLUS, BIOTECHNO, PASCAL, DGENE, PROMT, ESBIOBASE, IFIPAT, TOXCENTER' ENTERED AT 14:40:39 ON 24 MAY 2006

L2 2045 S L1 AND HUMAN

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L3          419 S L2 AND RECOMBINANT
L4           93 S L3 AND PURIF?
L5           19 S L4 AND 99%
L6           16 DUP REM L5 (3 DUPLICATES REMOVED)

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=> d 16 ibib ab 1-16

L6 ANSWER 1 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN
AN 11091341 IFIPAT;IFIUDB;IFICDB
TITLE: METHODS FOR PRODUCING AND **PURIFYING** **RECOMBINANT** ALPHA-L-**IDURONIDASE**
INVENTOR(S): Chan; Wai-Pan, Castro Valley, CA, US
Chen; Lin, San Francisco, CA, US
Fitzpatrick; Paul A., Albany, CA, US
Henstrand; John M., Davis, CA, US
Kakkis; Emil D., Novato, CA, US
Qin; Minmin, Pleasanton, CA, US
Starr; Christopher M., Sonoma, CA, US
Wendt; Dan J., Walnut Creek, CA, US
Zecherle; Gary N., Novato, CA, US
PATENT ASSIGNEE(S): Unassigned
AGENT: MARSHALL, GERSTEIN & BORUN LLP, 233 S. WACKER DRIVE, SUITE 6300, SEARS TOWER, CHICAGO, IL, 60606, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2006040348	A1	20060223
APPLICATION INFORMATION:	US 2003-722371		20031124

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION OF:	US 2001-993038	20011113	ABANDONED
FAMILY INFORMATION:	US 2006040348	20060223	

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION

PARENT CASE DATA:

This application claims priority to U.S. application Ser. No. 09/ 711,202, filed Nov. 9, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/439,923, filed Nov. 12, 1999.

NUMBER OF CLAIMS: 28
AB The present invention provides a **recombinant human** alpha -Liduronidase and biologically active fragments and muteins thereof with a purity greater than **99%**. The present invention further provides large-scale methods to produce and **purify** commercial grade **recombinant human** alpha -L-**iduronidase** enzyme thereof.

L6 ANSWER 2 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN
AN 04404900 IFIPAT;IFIUDB;IFICDB
TITLE: **RECOMBINANT** ALPHA-L-**IDURONIDASE**, METHODS FOR PRODUCING AND **PURIFYING** THE SAME AND METHODS FOR TREATING DISEASES CAUSED BY DEFICIENCIES THEREOF
INVENTOR(S): Kakkis; Emil D., 2572 Laguna Vista Dr., Novato, CA, 94949, US
Tanamachi; Becky, 3343 Walnut Ave., Signal Hill, CA, 90809, US
PATENT ASSIGNEE(S): Unassigned
PRIMARY EXAMINER: Rao, Manjunath N
AGENT: Marshall, Gerstein & Borun LLP

	NUMBER	PK	DATE
PATENT INFORMATION:	US 7041487	B2	20060509
	US 2003013179	A1	20030116
APPLICATION INFORMATION:	US 2002-206443		20020725
EXPIRATION DATE:	12 Nov 2019		

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION OF:	US 1999-439923	19991112	6426208
FAMILY INFORMATION:	US 7041487	20060509	
	US 6426208		

DOCUMENT TYPE: Utility
Granted Patent - Utility, with Pre-Grant Publication
FILE SEGMENT: CHEMICAL
GRANTED

PARENT CASE DATA:

This application is a continuation application of U.S. patent application Ser. No. 09/439,923, filed Nov. 12, 1999, now U.S. Pat. No. 6,426,208, issued Jul. 30, 2002, which is incorporated herein by reference.

NOTE: Subject to any Disclaimer, the term of this patent is extended or adjusted under 35 USC 154(b) by 326 days. This Patent is subject to a Terminal Disclaimer.
NUMBER OF CLAIMS: 33
GRAPHICS INFORMATION: 11 Drawing Sheet(s), 15 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1 represents the nucleotide and deduced amino acid sequences of cDNA encoding alpha-L-**iduronidase** (SEQ ID Nos: 1 and 2). Nucleotides 1 through 6200 are provided. Amino acids are provided starting with the first methionine in the open reading frame.
FIG. 2 represents the results from an SDS-PAGE run of eluate obtained according to the procedure set forth in Example 1. Lane 1 is blank. Lane 2 contained high molecular weight standards. Lane 3 is a blank. Lane 4 contained bovine serum albumin in a concentration of 50 mu g. Lanes 5 through 10 represent eluate containing recombinantly produced **human** alphaL-**iduronidase** in amounts of 1 mu g, 2 mu g, 5 mu g, 5 mu g, 5 mu g and 5 mu g, respectively.
FIG. 3 reveals the urinary GAG levels in 16 MPS I patients in relation to normal excretion values. There is a wide range of urine GAG values in untreated MPS I patients. A greater than 50% reduction in excretion of undegraded GAGs following therapy with **recombinant** alpha-L-**iduronidase** is a valid means to measure an individual's response to therapy.
FIG. 4 demonstrates leukocyte **iduronidase** activity before and after enzyme therapy in MPS I patients.
FIG. 5 demonstrates the buccal **iduronidase** activity before and after enzyme therapy.
FIG. 6 demonstrates in three patients that a substantial shrinkage of liver and spleen together with significant clinical improvement in joint and soft tissue storage was associated with a greater than 65% reduction in undegraded GAG after only 8 weeks of treatment with **recombinant** enzyme.
FIG. 7 demonstrates that there is substantial normalization of livers (FIG. 7A) and spleens (FIG. 7B) in patients treated with **recombinant** enzyme after only 12 weeks of therapy.
FIG. 8 demonstrates a precipitous drop in urinary GAG excretion over 22 weeks of therapy with **recombinant** enzyme in 6 patients.

AB The present invention provides a **recombinant** alpha -Liduronidase and biologically active fragments and mutants thereof, methods to produce and **purify** this enzyme as well as methods to treat certain genetic disorders including- alpha -Liduronidase deficiency and mucopolysaccharidosis I (MPS 1).

L6 ANSWER 3 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN

AN 10982027 IFIPAT;IFIUDB;IFICDB

TITLE: VIRAL VECTORS AND METHODS FOR PRODUCING AND USING THE SAME

INVENTOR(S): Amalfitano; Andrea, Durham, NC, US
Koeberl; Dwight D., Durham, NC, US
Sun; Baodong, Morrisville, NC, US

PATENT ASSIGNEE(S): Unassigned

AGENT: JENKINS, WILSON & TAYLOR, P. A., 3100 TOWER BLVD, SUITE 1400, DURHAM, NC, 27707, US

	NUMBER	PK	DATE	
PATENT INFORMATION:	US 2005220766	A1	20051006	
APPLICATION INFORMATION:	US 2003-511980		20030430	
	WO 2003-US13323		20030430	
			20050407	PCT 371 date
			20050407	PCT 102(e) date

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	US 2002-376397P	20020430	(Provisional)
FAMILY INFORMATION:	US 2005220766	20051006	

DOCUMENT TYPE: Utility
Patent Application - First Publication

FILE SEGMENT: CHEMICAL
APPLICATION

GOVERNMENT INTEREST:
This work was supported by grant R01-DK 52925 from the U.S. National Institute of Health. Thus, the U.S. government has certain rights in the invention.

PARENT CASE DATA:

This application is based on and claims priority to U.S. Provisional Application Ser. No. 60/376,397, filed Apr. 30, 2002, herein incorporated by reference in its entirety.

NUMBER OF CLAIMS: 142 17 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1A is a schematic of a hybrid Ad-AAV vector containing the chicken beta-actin (CB) promoter driving the hGAA cDNA. The hybrid vector, AdAAVCBGAApA, was constructed by bacterial recombination. The packaging size for the AAV vector sequence is 4.4 kb.

FIG. 1B is an autoradiograph depicting Southern blot analysis of DNase I-resistant hybrid Ad-AAV vector particles (Ad-AAV hybrid) , the plasmid containing the AAV vector sequences prior to bacterial recombination to produce Ad particles (pShuttle-AAV), and the AAV vector plasmid (pAAVCBGAApA). DNA was analyzed with AhdI and BssHII to determine that the AAV TR sequences were present (not deleted during recombination). AhdI cuts once in each terminal repeat and BssHII cuts twice in each terminal repeat, and each restriction digest gives unique fragments that were present in the **recombinant** Ad-AAV DNA.

FIG. 1C is a photograph of a cesium chloride gradient of hybrid Ad-AAV vector particles. Two viral bands were present, which eqilibrated at positions below a layer of protein at the top of the gradient.

FIG. 1D is an autoradiograph depicting Southern blot analysis of the two viral bands in FIG. 1C. Vector DNA was treated with DNase I and extracted prior to restriction enzyme analyis and Southern blotting. Each sample was 10 mu l. Lanes (11)-(16) contain linearized Ad5-containing plasmid representing the indicated number of double-stranded (ds) Ad particles. Therefore, the vector stock **purified** from the lower band contained 3.1x1011 DNase

I-resistant Ad-AAV vector particles per ml (lanes 6-9).
FIG. 1E is a schematic of a hybrid Ad-AAV vector packaging method for AAV vector **purification**. 293 cells were transfected with split AAV helper plasmids and transduced with a hybrid AdAAV vector containing the AAV vector sequences. No contaminating modified Ad vector is replicated by 293 cells (Amalfitano et al. (1998) J. Virol. 72:926-933). The AAV vector was ***purified*** by heparin-agarose column method (Zolotukhin et al. (1999) Gene Ther. 6:973-985).
FIG. 2A is an autoradiograph of a Southern blot depicting AAV vector packaging with an Ad-AAV hybrid vector. For the transfection-only method, 293 cells were transfected with plasmids containing the AAV rep and cap genes driven by heterologous promoters (Allen et al. (2000) Mol. Ther. 1:88-95) and with the AAV vector plasmid and pLNCorf6 (Scaria et al. (1995) Gene Ther. 2:295-298) (lane 1 only). For the hybrid AdAAV method of AAV vector packaging, the cells were transduced with the indicated number of hybrid Ad-AAV vector DNase-I resistant particles, and transfected with plasmids containing the AAV rep and cap genes (as shown in FIG. 1D). The Southern blot shows the yield of DNase I-resistant single-stranded AAV vector genomes per cell for each condition. Each sample represented 6x105 293 cells. Lanes (7) to (11) contained vector plasmid, digested with BgIII to release the double-stranded AAV vector sequences, representing the indicated number of singlestranded (ss) AAV vector particles.
FIG. 2B is a bar graph showing that AAV-CBGAApA was packaged with different Ad and AAV helpers. Five conditions for packaging of AAV-CBGAApA were evaluated, including transfection of pAAV-CBGAApA plus split AAV helper plasmids and pLNCorf6 (adapted from Allen et al., 2000), hybrid Ad-AAV transduction plus transfection of split AAV helper plasmids, modified Ad ((E1-,polymerase-)AdCMVLacZ) transduction plus split transfection of split AAV helper plasmids, wild-type Ad5 infection plus tranfection of pACG2 (Xiao et al. (1998) J. Virol. 72:10222-10226) and pAAV-CBGAApA, and hybrid Ad-AAV transduction plus transfection of pACG2.
FIG. 2C is an autoradiograph of a Southern blot that was performed to quantify the contaminating Ad-AAV genomes. Lanes as follows: (1) untreated 293 cells, (2) transfection of pMVCBGAApA plus split AAV helper plasmids and pLNCorf6 (adapted from Allen et al. 2000), (3) hybrid Ad-AAV transduction plus transfection of split AAV helper plasmids, (4) modified Ad ((E1,polymerase-)AdCMVLacZ) transduction plus split transfection of split AAV helper plasmids, (5) wild-type Ad5 infection plus tranfection of pACG2 (Xiao et al. (1998) J. Virol. 72:1022210226) and pAAV-CBGAApA, and (6) hybrid Ad-AAV transduction plus transfection of pACG2, (7) no sample, and (8)-(12) linearized Ad5-containing plasmid representing the indicated number of double-stranded (ds) Ad particles. Each sample represented 6x105 293 cells.
FIG. 3A is a bar graph depicting analysis of large-scale AAV vector packaging with an Ad-AAV hybrid vector. The yield of DNase I-resistant AAV vector particles for AAV-CBGAApA packaged by transfection of pLNCorf6, or transduction with the Ad-AAV hybrid to provide Ad helper functions, compared to a vector encoding glucose-6-phosphatase, AAV-CBcG6PpA, packaged with pLNCorf6. Twenty to 40 plate vector preparations were **purified** (3 vector preparations per condition), and the yield was calculated per cell plated. The mean number of AAV vector particles per cell is shown with the standard deviation indicated.
FIG. 3B is an autoradiograph of a Southern blot analysis of AAVCBGAApA ***purification*** , quantified versus titrated vector plasmid DNA. The samples represent vector DNA extracted from 25 microliters of sample. Standard amounts of vector plasmids were loaded for quantitation of vector particles. Lanes represent the following samples: (1) Crude cell lysate, (2) 40% iodoxinal fraction (3) Heparin-agarose (HA) column flow-through, (4) HA column wash, (5) HA column eluate fraction (ef) 1, (6) HA column ef 2, (7) HA column ef 3, (8) HA column ef 4, (9) HA column ef 2 after dialysis (10) HA column ef 2 plus 2.5x1010 particles AAV vector plasmid, (11) HA column ef 2 plus 2.5x1010 particles AAV vector plasmid, no DNase I added, (12)-(18) vector plasmid representing the indicated number of singlestranded (ss) AAV vector particles. Therefore, the **purified** AAV vector stock contained 4.8x1011 DNase I-resistant vector particles per ml.

FIG. 3C is an autoradiograph of a Southern blot analysis that quantitated the contaminating Ad-AAV genomes in the samples described in FIG. 3B above. Lanes 10-18 differed as follows: (10) HA column ef 2 plus 2.5x109 particles Ad-containing plasmid, (11) HA column ef 2 plus 2.5x109 particles Adcontaining plasmid, no DNase I added, (12) No sample, (13)-(17) linearized Ad5-containing plasmid representing the indicated number of double-stranded (ds) Ad particles. The residual AdAAV in the AAV vector stock was reduced to less than 1 infectious particle per 1010 AAV vector particles.
FIG. 4A depicts Western blot analysis of plasma that was performed at 3 days following intravenous administration of the hybrid Ad-AAV (essentially Ad) vector encoding hGAA (2x1010 vector particles/mouse). **Recombinant** hGAA (rhGAA) is shown for reference (2 ng total), and the 110 kD hGAA precursor was detected as expected (Amalfitano et al. (1999) Proc. Natl. Acad. Sci. U.S.A. 96:8861-8866, Ding et al. (2001) Hum. Gene Ther. 12:955-965).
FIG. 4B depicts Western blot analysis of GAA-KO mice that received a hybrid Ad-AAV vector (2x1010 DNase I-resistant vector particles) or an AAV vector (4x1010 DNase I-resistant vector particles or 1x1012 DNase I-resistant vector particles) encoding hGAA by intravenous administration. Western blot analysis of liver is shown at 2 and 6 weeks after vector administration for each group (n=3 for each group). (Note: hGAA in mouse liver migrates slightly faster than rhGAA.) GAA-KO mice that received an AAV vector (1x1012 vector particles) encoding hGAA by intravenous administration (n=2) shown 6 weeks after vector administration. Untreated, affected GAA-KO mouse liver is shown for comparison (No vector, n=2). For the higher number of AAV vector particles, the 67 kD, 76 kD, and 110 kD hGAA species were detected as expected (Amalfitano et al. (1999) Proc. Nat. Acad. Sci. U.S.A. 96:8861-8866, Ding et al. (2001) Hum. Gene Ther. 12:955-965).
FIGS. 5A-5C depict **human** GAA secretion and uptake following portal vein injection of an AAV vector in GAA-KO mice.
FIG. 5A depicts Western blot analysis of plasma from GAAKO SCID mice at the indicated times following portal vein injection of the indicatedAAV vector encoding hGAA, and from untreated, GAAKO SCID mice (Controls). Each lane represents an individual mouse.
FIG. 5B is a bar graph that summarizes GAA analysis for tissues following portal vein injection of an AAV vector. GAA-KO/SCID mice received the vector packaged as AAV2 (n=1) or AAV6 (n=1). Controls were age-matched, untreated GAA-KO/SCID mice (n=2). The GM level was analyzed twice, independently, and the average and range are shown.
FIG. 5C is a photomicrograph depicting periodic acid Schiff (PAS) staining of the heart for a GAA-KO/SCID mouse that received an AAV vector (left panel AAV-CBGAApA) and for an untreated GAA-KO/ SCID mouse (right panel) and HE staining (lower panels). Magnification 100x.

AB A **recombinant** hybrid virus, including: (a) a deleted adenovirus vector genome comprising the adenovirus 5' and 3' cis-elements for viral replication and encapsidation, and further comprising a deletion in an adenovirus genomic region selected from the group consisting of: (i) the polymerase region, wherein said deletion essentially prevents the expression of a functional polymerase protein from said deleted region and said hybrid virus does not otherwise express a functional polymerase protein, (ii) the preterminal protein region, wherein said deletion essentially prevents the expression of a functional preterminal protein from said deleted region, and said hybrid virus does not otherwise express a functional preterminal protein, and (iii) both the regions of (i) and (ii); and (b) a **recombinant** adeno-associated virus (AAV) vector genome flanked by the adenovirus vector genome sequences of (a), said **recombinant** AAV vector genome comprising (i) AAV 5' and 3' inverted terminal repeats, (ii) an AAV packaging sequence, and (iii) a heterologous nucleic acid sequence, wherein said heterologous nucleic acid sequence is flanked by the 5' and the 3' AAV inverted terminal repeats of (i). Methods of making and using the **recombinant** hybrid virus are also disclosed.

AN 10919552 IFIPAT;IFIUDB;IFICDB
TITLE: METHODS AND COMPOSITIONS FOR THE PRODUCTION OF ADENOVIRAL VECTORS
INVENTOR(S): Pham; Hai, Houston, TX, US
Zhang; Shuyuan, Sugar Land, TX, US
PATENT ASSIGNEE(S): Unassigned
AGENT: FULBRIGHT & JAWORSKI L.L.P., 600 CONGRESS AVE., SUITE 2400, AUSTIN, TX, 78701, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2005158283	A1	20050721
APPLICATION INFORMATION:	US 2005-79986		20050315

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
DIVISION OF:	US 2003-439278	20030515	PENDING
FAMILY INFORMATION:	US 2005158283	20050721	

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION

NUMBER OF CLAIMS: 62 7 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1A show results of a study demonstrating the effect of temperature on Adp53 production (vp/flask).
FIG. 1B shows results of a study demonstrating the effect of temperature on Admda7 production (vp/flask).
FIG. 2. Cell growth and viability in the bioreactor.
FIG. 3. Glucose and lactate concentrations (g/L) in media vs. days in culture.
FIG. 4. Diagram of a perfusion bioreactor system.
FIG. 5. Cell growth and viability in perfusion culture vs. days in culture.
FIG. 6. Glucose and lactate concentrations (g/L) in perfusion culture vs. days in culture.

AB The present invention addresses the need to improve the yield of adenovirus when grown in cell culture systems. In particular, it has been demonstrated that for adenovirus, the use of infection temperatures lower than 37 degrees C. in a cell culture system results in improved yields of adenovirus. In addition, it has been demonstrated that when host cells are grow in a bioreactor, initiating adenovirus infection by diluting the host cells with fresh media and adenovirus results in improved yield of adenovirus. Methods of adenoviral production and **purification** using infection temperatures less than 37 degrees C. are disclosed. Methods of adenoviral production and **purification** wherein the host cells are grown in a bioreactor and adenovirus infection is initiated by diluting the host cells with fresh media and adenovirus are also disclosed.

L6 ANSWER 5 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN
AN 04202137 IFIPAT;IFIUDB;IFICDB
TITLE: METHODS FOR TREATING DISEASES CAUSED BY DEFICIENCIES OF **RECOMBINANT** ALPHA-L-**IDURONIDASE**
INVENTOR(S): Kakkis; Emil D., 2512 Laguna Vista Dr., Novato, CA, 94949, US
PATENT ASSIGNEE(S): Unassigned
PRIMARY EXAMINER: Rao, Manjunath N
AGENT: Marshall, Gerstein & Borun LLP

	NUMBER	PK	DATE
PATENT INFORMATION:	US 6858206	B2	20050222

US 2002164758 A1 20021107
APPLICATION INFORMATION: US 2001-993241 20011113
EXPIRATION DATE: 12 Nov 2019

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION-IN-PART OF:	US 1999-439923	19991112	6426208
CONTINUATION-IN-PART OF:	US 2000-711205	20001109	6585971

FAMILY INFORMATION: US 6858206 20050222
US 6426208
US 6585971
US 2002164758 20021107
DOCUMENT TYPE: Utility
Granted Patent - Utility, with Pre-Grant Publication
FILE SEGMENT: CHEMICAL
GRANTED

PARENT CASE DATA:

This application is a continuation-in-part to U.S. patent application Ser. No. 09/711,205, filed on Nov. 9, 2000 now U.S. Pat. No. 6,585,971, Jul. 1, 2003 which is a continuation-inpart of U.S. patent application Ser. No. 09/439,923, filed on Nov. 12, 1999.

NOTE: Subject to any Disclaimer, the term of this patent is extended or adjusted under 35 USC 154(b) by 203 days.
MICROFILM REEL NO: 014767 FRAME NO: 0890
014770 0226
014772 0436
015398 0139
015577 0341
015580 0887
015584 0474
NUMBER OF CLAIMS: 46
GRAPHICS INFORMATION: 14 Drawing Sheet(s), 20 Figure(s).
DESCRIPTION OF FIGURES:

FIG. 1 represents the nucleotide and deduced amino acid sequences of cDNA encoding alpha-L-**iduronidase** (SEQ ID NOS:1 and 2). Nucleotides 1 through 6200 are provided. Amino acids are provided starting with the first methionine in the open reading frame.

FIG. 2 represents the results from SDS-PAGE runs of eluate obtained according to the procedures as described below. The top panel shows the SDS-PAGE results of **purified** alpha-Liduronidase (3 micrograms) and contaminants from the production/ **purification** scheme disclosed in Kakkis, et al., Protein Expr. **Purif**. 5: 225-232 (1994). In the bottom panel, SDS-PAGE results of **purified** alpha-L-**iduronidase** with contaminants from an unpublished prior production/**purification** process (U.S. patent application Ser. Nos. 09/078,209 and 09/170,977) referred to as the Carson method in Lanes 2 (7.5 microgram alpha-Liduronidase) and Lane 3 (5.0 microgram alpha-L-**iduronidase**) are compared to that of the production/**purification** process of the present invention referred to as the Galli Process (Lane 4 5 micrograms alpha-L-**iduronidase**). Lane 1 contains the molecular weight marker. FIG. 2 shows that the Galli production/ ***purification*** method of the present invention yields a highly ***purified*** alpha-L-**iduronidase** product with fewer contaminants in comparison with prior production/**purification** schemes.

FIG. 3 demonstrates the alpha-**iduronidase** production level over a 30-day period, during which time cells are switched at day 5 from a serum containing medium to a serum-free medium. alphaIduronidase production was characterized by: (1) absence of a need for adaptation when cells are switched from serumcontaining to serum-free medium at 100200 (top and bottom panels) with an uninterrupted increase in productivity (top panel); (2) a high level of

production in excess of 4 mg per liter (1000 per mL) in a protein-free medium (bottom panel); and (3) a boost in alpha-**iduronidase** production with butyrate induction events (bottom panel).
FIG. 4 demonstrates a decrease in liver volume during enzyme therapy in MPS I patients.
FIG. 5 demonstrates urinary GAG excretion during enzyme therapy.
FIG. 6 demonstrates elbow and knee extension in HAC002 during enzyme therapy.
FIG. 7 demonstrates shoulder flexion to 104 weeks in four patients with the most restriction during enzyme therapy.
FIG. 8 demonstrates improvement in sleep apnea before and after six weeks of therapy.
FIG. 9 demonstrates the improvement in apneas and hypopneas during sleep with enzyme therapy in each individual patient.
FIG. 10 demonstrates the improvement in pulmonary function tests before and after 12 and 52 weeks of enzyme therapy in one patient.
FIG. 11 demonstrates increased height growth velocity with enzyme therapy.
FIG. 12 shows the degree of contamination by Chinese Hamster Ovary Protein (CHOP) and degree of purity of alpha-Liduronidase, produced by (1) the Carson method, an unpublished prior production/**purification** process (U.S. patent application Ser. Nos. 09/078,209 and 09/170,977 and (2) the Galli method, the production/**purification** process of the present invention. Thus,
FIG. 12 shows that alpha-L-**iduronidase** produced and **purified** by the Galli method has a higher degree of purity and lower degree of CHOP contamination in comparison to that of the Carson method.
FIG. 13 shows a comparison of alpha-L-**iduronidase** produced by the Galli method versus the Carson method. On the left side of the Figure, results from a Western Blot show that the Galli material (left side, column 2) comprise fewer contaminating protein bands (between 48 kDa and 17 kDa) in comparison with the Carson material (left side, column 3). On the right side of the Figure, results from an SDS-PAGE silver stain show the absence of a band at the 62 kDa in the Galli material (column 2) in comparison to the presence of such a band in the Carson material (column 3).
AB The present invention provides a formulation comprising a pharmaceutical composition comprising a **human recombinant** alpha -L-**iduronidase** or biologically active or muteins thereof with a purity of greater than **99%**, or in combination with a pharmaceutically acceptable carrier. The present invention further provides methods to treat certain genetic disorders including alpha -L-**iduronidase** deficiency and mucopolysaccharidosis I (MPS 1) by administering said formulation.

L6 ANSWER 6 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN

AN	10722085 IFIPAT;IFIUDB;IFICDB
TITLE:	METHODS AND COMPOSITIONS FOR THE PRODUCTION OF ADENOVIRAL VECTORS
INVENTOR(S):	Pham; Hai, Houston, TX, US Zhang; Shuyuan, Sugar Land, TX, US
PATENT ASSIGNEE(S):	Introgen Therapeutics, Inc., US
AGENT:	FULBRIGHT & JAWORSKI L.L.P., 600 CONGRESS AVE., SUITE 2400, AUSTIN, TX, 78701, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2004229335	A1	20041118
APPLICATION INFORMATION:	US 2003-439278		20030515
FAMILY INFORMATION:	US 2004229335		20041118

DOCUMENT TYPE:	Utility Patent Application - First Publication
FILE SEGMENT:	CHEMICAL APPLICATION
OTHER SOURCE:	CA 142:5560

NUMBER OF CLAIMS: 80 7 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1A show results of a study demonstrating the effect of temperature on Adp53 production (vp/flask).
FIG. 1B shows results of a study demonstrating the effect of temperature on Admda7 production (vp/flask).
FIG. 2. Cell growth and viability in the bioreactor.
FIG. 3. Glucose and lactate concentrations (g/L) in media vs. days in culture.
FIG. 4. Diagram of a perfusion bioreactor system.
FIG. 5. Cell growth and viability in perfusion culture vs. days in culture.
FIG. 6. Glucose and lactate concentrations (g/L) in perfusion culture vs. days in culture.

AB The present invention addresses the need to improve the yield of adenovirus when grown in cell culture systems. In particular, it has been demonstrated that for adenovirus, the use of infection temperatures lower than 37 degrees C. in a cell culture system results in improved yields of adenovirus. In addition, it has been demonstrated that when host cells are grow in a bioreactor, initiating adenovirus infection by diluting the host cells with fresh media and adenovirus results in improved yield of adenovirus. Methods of adenoviral production and **purification** using infection temperatures less than 37 degrees C. are disclosed. Methods of adenoviral production and **purification** wherein the host cells are grown in a bioreactor and adenovirus infection is initiated by diluting the host cells with fresh media and adenovirus are also disclosed.

L6 ANSWER 7 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN

AN	10654604 IFIPAT;IFIUDB;IFICDB		
TITLE:	METHODS OF **PURIFYING HUMAN** ACID ALPHA-GLUCOSIDASE		
INVENTOR(S):	van der Ploeg; Ans T., Poortugaal, NL Reuser; Arnold J., Rotterdam, NL		
PATENT ASSIGNEE(S):	Unassigned		
AGENT:	Gary M. Nath;NATH & ASSOCIATES PLLC, 6th Floor, 1030 15th Street, N. W., Washington, DC, 20005, US		

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2004161837	A1	20040819
	US 6118045	A1	
	ORIGINAL PATENT		
APPLICATION INFORMATION:	US 2004-777644		20040213
	US 1996-700760		19960729
	ORIGINAL APPLICATION		

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION-IN-PART OF:	US 2001-770253	20010129	ABANDONED
CONTINUATION-IN-PART OF:	US 2002-46180	20020116	PENDING
DIVISION OF:	US 2001-886477	20010622	ABANDONED

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	US 1995-1796P	19950802	(Provisional)
FAMILY INFORMATION:	US 2004161837	20040819	
	US 6118045		
DOCUMENT TYPE:	Utility Patent Application - First Publication		
FILE SEGMENT:	CHEMICAL APPLICATION		
OTHER SOURCE:	CA 141:186901		

PARENT CASE DATA:

The present application is a continuation-in-part of U.S. patent application Ser. No. 09/770,253 filed Jan. 29, 2001 which is a continuation-in-part of U.S. patent application Ser. No. 60/001, 796 filed Aug. 2, 1995, which is now U.S. Pat. No. 6,118,045, granted Sep. 12, 2000 examined as U.S. patent application Ser. No. 08/700,760 filed Jul. 29, 1996 the subject matter of each incorporated by reference herein in their entirety and a continuation-in-part of U.S. patent application Ser. No. 60/111, 291 filed Dec. 7, 1998, which is now published as WO/00/34451 on Jun. 15, 2000 from PCT application US99/29042, filed Dec. 6, 1999 the subject matter of each incorporated by reference herein in their entirety.

NUMBER OF CLAIMS: 39 18 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1: A transgene containing acid α-glucosidase cDNA. The αs1-casein exons are represented by open boxes; . alpha.-glucosidase cDNA is represented by a shaded box. The . alpha.s1-casein intron and flanking sequences are represented by a thick line. A thin line represents the IgG acceptor site. The transcription initiation site is marked (), the translation initiation site (ATG), the stopcodon (TAG) and the polyadenylation site (pA).

FIG. 2 (panels A, B, C): Three transgenes containing acid α -glucosidase genomic DNA. Dark shaded areas are αs1 casein sequences, open boxes represent acids αglucosidase exons, and the thin line between the open boxes represents α-glucosidase introns. Other symbols are the same as in FIG. 1.

FIG. 3 (panels A, B, C): Construction of genomic transgenes. The α-glucosidase exons are represented by open boxes; the . alpha.-glucosidase introns and nontranslated sequences are indicated by thin lines. The pKUN vector sequences are represented by thick lines.

FIG. 4 (panels A and B). Detection of acid α-glucosidase in milk of transgenic mice by Western blotting.

FIG. 5. Chromatography profile of rabbit whey on a Q Sepharose FF column.

FIG. 6. Chromatography profile of Q Sepharose FF-**purified** ***recombinant*** **human** a-glucosidase fraction on a Phenyl HP Sepharose column.

FIG. 7. Chromatography profile of a (Phenyl HP Sepharosepurified) ***recombinant*** **human** a-glucosidase fraction on Source Phenyl 15 column.

FIG. 8. SDS-PAGE analysis of various fractions during the acid aglucosidase ***purification*** procedure. Various fractions obtained during a ***recombinant*** **human** acid a-glucosidase **purification** from rabbit milk (line 60) were diluted in non-reduced SDS sample buffer. The samples were boiled for 5 minutes and loaded on a SDS-PAGE gradient gel (4-12%, Novex).

FIG. 9. HPLC size exclusion profile of **purified recombinant** ***human*** acid aglucosidase precursor.

FIG. 10. Binding of 1251 **human** acid a-glucosidase precursor to various metal-chelating and lectin Sepharoses. **Purified human** acid a-glucosidase precursor from rabbit line 60 was radiolabeled with 1251 as described in Example 5. Binding of the labeled enzyme to the metal-chelating Sepharoses (Fe2+, Fe3+, Cu2+, Zn2+, glycine, and control) and to the lectin Sepharoses (Concanavalin A and lentil) was done as described in Example 1. Two washing procedures were tested: either a wash with PBS, 0. 002% Tween-20 buffer, or a wash with PBS, 0.1% Tween-20,0.5 M sodium chloride buffer. The binding percentages relate to the total amount of radiolabel added to the tubes.

FIG. 11. Chromatographic elution profiles of acid a-glucosidasecontaining fractions on various HIC columns.

FIG. 12 Chromatography profiles of transgenic and non-transgenic whey fractions on a Hydroxylapatite column. Transgenic (----) and non-transgenic (-.-.-.-) rabbit whey, obtained after skimming (by centrifugation) and casein removal (by TFF), were loaded on a Amberchrome column (4.6x150 mm) containing MacroPrep

ceramic hydroxylapatite type I (40 Ltm beads; BioRad) connected to a FPLC system of Pharmacia. Whey fractions obtained after TFF were diluted 5-fold in buffer A (10 mM NaPi pH 6.8), and 0.2 ml was loaded on the column pre-equilibrated in buffer A. The flow rate was 2 ml/min. After loading, bound protein was eluted with a gradient to 500 mM NaPj pH 6.8 in 10 column volumes. Protein was detected by measuring the absorbance at 280 nm (flow cell is 2 mm).
FIG. 13. SDS-PAGE analysis of whey fractions from the hydroxylapatite column. Transgenic and non-transgenic rabbit whey were loaded on the Macro-Prep ceramic hydroxylapatite type) column as described in FIG. 12.
FIGS. 14 to 19 are chromatograms of hydroxylapatite chromatography separations of transgenic whey samples in which the samples were loaded on to the column at sodium phosphate buffer (NaPi) concentrations of 5, 10, 20, 30, 40 or 50 mM respectively. The pH of the buffer was 7.0. The chromatograms show the gradient of sodium phosphate eluting buffer to 400 mM, the AZSO and the pH of the eluate and the fractions collected.
FIGS. 20 to 23 are chromatograms of hydroxylapatite chromatography separations as in FIGS. 14 to 19 above except that the pH of the sample was varied whilst the NaPi buffer concentration was retained at 5 mM. The pH of the samples fractionated were pH 6.0, 7.0 and 7.5 respectively.
FIG. 24 is a chromatogram of an industrial (pilot) scale separation of transgenic milk whey on Q Sepharose FF.
FIG. 25 is a chromatogram of hydroxylapatite column chromatography of 0.1 M eluate from the Q Sepharose FF column.
FIG. 26 is a silver stained SDS-PAGE gel of flow through fractions from a series of hydroxylapatite chromatography separations of 0.1 M eluates of Q Sepharose FF.
AB The invention provides methods of **purifying** lysosomal proteins, pharmaceutical compositions for use in enzyme replacement therapy, and methods of treating Pompe's disease using **purified** **human** acid alpha glucosidase.

L6 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2003:408803 CAPLUS
DOCUMENT NUMBER: 139:5755
TITLE: Large scale production and **purification** of **human recombinant** α-L-**iduronidase** for treating mucopolysaccharidosis I
INVENTOR(S): Qin, Minmin; Chan, Wai-pan; Chen, Lin; Fitzpatrick, Paul A.; Hendstrand, John M.; Wendt, Dan J.; Zecherle, Gary N.; Starr, Christopher M.; Kakkis, Emil D.
PATENT ASSIGNEE(S): Biomarin Pharmaceutical Inc., USA
SOURCE: U.S., 34 pp., Cont.-in-part of U.S. Ser. No. 439,923. CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6569661	B1	20030527	US 2000-711202	20001109
US 6426208	B1	20020730	US 1999-439923	19991112
WO 2002038775	A2	20020516	WO 2001-US47843	20011109
WO 2002038775	A3	20040226		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR,

IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002027369	A5	20020521	AU 2002-27369	20011109
US 2002146802	A1	20021010	US 2001-993038	20011113
ZA 2002003619	A	20030507	ZA 2002-3619	20020507
US 2003013179	A1	20030116	US 2002-206443	20020725
US 7041487	B2	20060509		
US 2006040348	A1	20060223	US 2003-722371	20031124
JP 2005046154	A2	20050224	JP 2004-242343	20040823

PRIORITY APPLN. INFO.:

US 1999-439923	A2	19991112
JP 2002-509470	A3	20001109
US 2000-711202	A	20001109
WO 2001-US47843	W	20011109
US 2001-993038	A1	20011113

AB The present invention provides a method to mass produce **human recombinant** α-L- **iduronidase** in large scale amts. with appropriate purity to enable large scale production for long term patient use of the enzyme therapy. The method comprises the steps of: (a) harvesting and filtering fluid obtained from a culture of Chinese hamster ovary cells transformed with nucleic acids encoding the **human recombinant** α-L- **iduronidase**; (b) adjusting the pH of the fluid to an acidic pH wherein any potential virus is inactivated and said **human recombinant** α-L- **iduronidase** is not harmed, followed by filtration through a 0.2 μ to 0.54 μ filter; (c) passing the fluid from step (b) through a Cibacron Blue dye interaction chromatog. column to capture the **human recombinant** α-L- **iduronidase**; (d) passing the fluid through a copper chelation chromatog. column to remove contaminating Chinese hamster ovary proteins; (e) passing the fluid through a Ph hydrophobic interaction chromatog. column to reduce residual leached Cibacron Blue dye and copper ions canned over from previous columns; and (f) concentrating and diafiltering the **purified human recombinant** α-L- **iduronidase**; wherein said purity of equal to or greater than .apprx.**99%** purity of **human recombinant** α-L- **iduronidase** is measured by μg of contaminating Chinese hamster ovary protein per mg of total protein. The **purified** com. grade **recombinant human** α-L- **iduronidase** can be used for treating genetic disorders including α-L- **iduronidase** deficiency and mucopolysaccharidosis I.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN

AN 10268777 IFIPAT;IFIUDB;IFICDB

TITLE: **RECOMBINANT** ALPHA-L-**IDURONIDASE**, METHODS FOR PRODUCING AND **PURIFYING** THE SAME AND METHODS FOR TREATING DISEASES CAUSED BY DEFICIENCIES THEREOF; ENZYMATIC POLYPEPTIDE FOR USE IN THE TREATMENT OF LYSOSOMAL STORAGE DISORDERS

INVENTOR(S): Kakkis; Emil D., Novato, CA, US
Tanamachi; Becky, Signal Hill, CA, US

PATENT ASSIGNEE(S): Unassigned

PATENT ASSIGNEE PROBABLE: Los Angeles Biomedical Res Inst at Harbor Ucla Medical Center (Probable)

AGENT: HOWREY SIMON ARNOLD & WHITE, LLP, BOX 34, 301 RAVENSWOOD AVE., MENLO PARK, CA, 94025, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003013179	A1	20030116
APPLICATION INFORMATION:	US 2002-206443		20020725

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION OF:	US 1999-439923	19991112	6426208
FAMILY INFORMATION:	US 2003013179	20030116	
	US 6426208		

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION

NUMBER OF CLAIMS: 20 8 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1 represents the nucleotide and deduced amino acid sequences of cDNA encoding alpha-L-**iduronidase** (SEQ ID Nos: 1 and 2). Nucleotides 1 through 6200 are provided. Amino acids are provided starting with the first methionine in the open reading frame.
FIG. 2 represents the results from an SDS-PAGE run of eluate obtained according to the procedure set forth in Example 1. Lane 1 is blank. Lane 2 contained high molecular weight standards. Lane 3 is a blank. Lane 4 contained bovine serum albumin in a concentration of 50 mu g. Lanes 5 through 10 represent eluate containing recombinantly produced **human** alphaL-**iduronidase** in amounts of 1 mu g, 2 mu g, 5 mu g, 5 mu g, 5 mu g and 5 mu g, respectively.
FIG. 3 reveals the urinary GAG levels in 16 MPS I patients in relation to normal excretion values. There is a wide range of urine GAG values in untreated MPS I patients. A greater than 50% reduction in excretion of undegraded GAGs following therapy with **recombinant** alpha-L-**iduronidase** is a valid means to measure an individual's response to therapy.
FIG. 4 demonstrates leukocyte **iduronidase** activity before and after enzyme therapy in MPS I patients.
FIG. 5 demonstrates the buccal **iduronidase** activity before and after enzyme therapy.
FIG. 6 demonstrates in three patients that a substantial shrinkage of liver and spleen together with significant clinical improvement in joint and soft tissue storage was associated with a greater than 65% reduction in undegraded GAG after only 8 weeks of treatment with **recombinant** enzyme.
FIG. 7 demonstrates that there is substantial normalization of livers and spleens in patients treated with **recombinant** enzyme after only 12 weeks of therapy.
FIG. 8 demonstrates a precipitous drop in urinary GAG excretion over 22 weeks of therapy with **recombinant** enzyme in 6 patients.
AB The present invention provides a **recombinant** alpha -Liduronidase and biologically active fragments and mutants thereof, methods to produce and **purify** this enzyme as well as methods to treat certain genetic disorders including- alpha -Liduronidase deficiency and mucopolysaccharidosis I (MPS 1).

L6 ANSWER 10 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN
AN 03902072 IFIPAT;IFIUDB;IFICDB
TITLE: **RECOMBINANT** ALPHA-L-**IDURONIDASE**, METHODS FOR PRODUCING AND **PURIFYING** THE SAME AND METHODS FOR TREATING DISEASE CAUSED BY DEFICIENCIES THEREOF
INVENTOR(S): Kakkis; Emil D., Novato, CA
PATENT ASSIGNEE(S): Harbor-UCLA Research and Education Institute, Torrance, CA, US
PRIMARY EXAMINER: Achutamurthy, Ponnathapu
ASSISTANT EXAMINER: Rao, Manjunath N
AGENT: Chiang Robin C.
Halluin Albert P.
Howrey Simon Arnold & White LLP

NUMBER	PK	DATE

PATENT INFORMATION:	US 6585971	B1	20030701
APPLICATION INFORMATION:	US 2000-711205		20001109
EXPIRATION DATE:	12 Nov 2019		

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION-IN-PART OF:	US 1999-439923	19991112	6426208
FAMILY INFORMATION:	US 6585971	20030701	
	US 6426208		

DOCUMENT TYPE: Utility
REASSIGNED
Granted Patent - Utility, no Pre-Grant Publication

FILE SEGMENT: CHEMICAL
GRANTED

OTHER SOURCE: CA 139:73769

PARENT CASE DATA:

This application is a continuation-in-part of U.S. patent application Ser. No. 09/439,923, filed on Nov. 12, 1999 now U.S. Pat. No. 6,426,208, Jul. 30, 2002.

NOTE: Subject to any Disclaimer, the term of this patent is extended or adjusted under 35 USC 154(b) by 232 days.

MICROFILM REEL NO: 011804 FRAME NO: 0640
013922 0668
013989 0436

NUMBER OF CLAIMS: 30

GRAPHICS INFORMATION: 14 Drawing Sheet(s), 19 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 represents the nucleotide and deduced amino acid sequences of cDNA encoding alpha-L-**iduronidase** (SEQ ID NOS:1 and 2). Nucleotides 1 through 6200 are provided. Amino acids are provided starting with the first methionine in the open reading frame.

FIG. 2 represents the results from SDS-PAGE runs of eluate obtained according to the procedures as described below. The top panel shows the SDS-PAGE results of **purified** alpha-Liduronidase (3 micrograms) and contaminants from the production/ **purification** scheme disclosed in Kakkis, et al., Protein Expr. **Purif**. 5: 225-232 (1994). In the bottom panel, SDS-PAGE results of **purified** alpha-L-**iduronidase** with contaminants from an unpublished prior production/**purification** process (U.S. patent application Ser. Nos. 09/078,209 and 09/170,977) referred to as the Carson method in Lanes 2 (7.5 microgram alpha-Liduronidase) and Lane 3 (5.0 microgram alpha-L-**iduronidase**) are compared to that of the production/**purification** process of the present invention referred to as the Galli Process (Lane 4 5 micrograms alpha-L-**iduronidase**). Lane 1 contains the molecular weight marker. FIG. 2 shows that the Galli production/ ***purification*** method of the present invention yields a highly ***purified*** alpha-L-**iduronidase** product with fewer contaminants in comparison with prior production/**purification** schemes.

FIGS. 3A-3B demonstrates the alpha-**iduronidase** production level over a 30-day period, during which time cells are switched at day 5 from a serum-containing medium to a serum-free medium. alpha-**Iduronidase** production was characterized by: (1) absence of a need for adaptation when cells are switched from serumcontaining to serum-free medium at 100200 (top and bottom panels) with an uninterrupted increase in productivity (top panel); (2) a high level of production in excess of 4 mg per liter (1000 per mL) in a protein-free medium (bottom panel); and (3) a boost in alpha- ***iduronidase*** production with butyrate induction events (bottom panel).

FIG. 4 demonstrates a decrease in liver volume during enzyme therapy in MPS I patients.

FIG. 5 demonstrates urinary GAG excretion during enzyme therapy.

FIG. 6 demonstrates elbow and knee extension in HAC002 during enzyme therapy.
FIG. 7 demonstrates shoulder flexion to 104 weeks in four patients with the most restriction during enzyme therapy.
FIG. 8 demonstrates improvement in sleep apnea before and after six weeks of therapy.
FIG. 9 demonstrates the improvement in apneas and hypopneas during sleep with enzyme therapy in each individual patient.
FIG. 10 demonstrates the improvement in pulmonary function tests before and after 12 and 52 weeks of enzyme therapy in one patient.
FIG. 11 demonstrates increased height growth velocity with enzyme therapy.
FIG. 12 shows the degree of contamination by Chinese Hamster Ovary Protein (CHOP) and degree of purity of alpha-Liduronidase, produced by (1) the Carson method, an unpublished prior production/**purification** process (U.S. patent application Ser. Nos. 09/078,209 and 09/170,977 and (2) the Galli method, the production/**purification** process of the present invention. Thus, FIG. 12 shows that alpha-L-**iduronidase** produced and ***purified*** by the Galli method has a higher degree of purity and lower degree of CHOP contamination in comparison to that of the Carson method.

AB The present invention provides a **recombinant human** alpha -Liduronidase and biologically active fragments and mutants thereof, large scale methods to produce and **purify** commercial grade **recombinant human** alpha -L-**iduronidase** enzyme as well as methods to treat certain genetic disorders including alpha -Liduronidase deficiency and mucopolysaccharidosis I (MPS 1).

L6 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2002:368665 CAPLUS
DOCUMENT NUMBER: 136:385047
TITLE: Methods for large scale production and **purification** of **human** α-L-**iduronidase** for treatment of mucopolysaccharidosis I
INVENTOR(S): Qin, Minmin; Chan, Wai-Pan; Chen, Lin; Fitzpatrick, Paul A.; Henstrand, John M.; Wendt, Dan J.; Zecherle, Gary N.; Starr, Christopher M.; Kakkis, Emil D.
PATENT ASSIGNEE(S): Biomarin Pharmaceutical, Inc., USA
SOURCE: PCT Int. Appl., 71 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002038775	A2	20020516	WO 2001-US47843	20011109
WO 2002038775	A3	20040226		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 6569661	B1	20030527	US 2000-711202	20001109
AU 2002027369	A5	20020521	AU 2002-27369	20011109

PRIORITY APPLN. INFO.:		
US 2000-711202	A	20001109
US 1999-439923	A2	19991112
WO 2001-US47843	W	20011109

AB The present invention provides a **recombinant human**

α-L- **iduronidase** and biol. active fragments and muteins thereof with a purity greater than **99%**. The present invention further provides large-scale methods to produce and **purify** com. grade **recombinant human** α-L- **iduronidase** enzyme thereof. The method involves preparation of a seed culture containing Chinese hamster ovary cells 2.131 transfected with a vector encoding cDNA for α-L- **iduronidase**. These cells is washed and resuspended in a protein-free culture medium supplemented with 7.6 mg/L thymidine, 13.6 mg/L hypoxanthine, 375 μg/mL G418 and 5% fetal bovine serum. The cell suspension is incubated at 37°C for 2-3 days with 5% CO2 in three 225 cm flasks. The said cell suspension is split by sequentially adding the cells to one 1L spinner flask, two 3L flasks and 4 8L flasks. The cell suspension is stirred at 50 rpm, followed by increasing the inoculum volume by incubating and subculturing cells to a final cell d. of about 2-2.5 x 105. A mixture containing macroporous microcarriers is prepared in growth medium with fetal bovine serum and transfering said mixture to a bioreactor. Cells from the bioreactor may be harvested at a d. of about 106. Methods for **purifn**. of α-L- **iduronidase** to greater than **99%** purity include adjusting the pH to an acidic range, followed by filtering the mixture through a 0.2-0.54 μ filter. The filtrate is further passed through a blue sepharose FF column to capture the protein which **purifies** α-L- **iduronidase** 7-10-fold. Contaminating CHO proteins are removed by passing the fluid through a copper chelating sepharose column. The mixture is then passed through a Ph sepharose column to reduce residual leached Cibacron blue dye and copper ions carried over from the previous columns. **Purified** α-L- **iduronidase** is concentrated and diafiltered. The **purifn**. steps include 10% glycerol in all buffers to improve the α-L- **iduronidase** yield. The specific activity of α-L- **iduronidase** may be greater than 240,000 units/mg protein.

L6 ANSWER 12 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN

AN	10221051 IFIPAT;IFIUDB;IFICDB
TITLE:	METHODS FOR TREATING DISEASES CAUSED BY DEFICIENCIES OF **RECOMBINANT** ALPHA-L-**IDURONIDASE** ; ENZYMATIC POLYPEPTIDE FOR USE IN THE DIAGNOSIS AND TREATMENT OF ALLERGIES, HEART AND RESPIRATORY DISORDERS
INVENTOR(S):	Kakkis; Emil D., Novato, CA, US
PATENT ASSIGNEE(S):	Unassigned
PATENT ASSIGNEE PROBABLE:	Los Angeles Biomedical Res Inst at Harbor Ucla Medical Center (Probable)
AGENT:	HOWREY SIMON ARNOLD & WHITE, LLP, BOX 34, 301 RAVENSWOOD AVE., MENLO PARK, CA, 94025, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002164758	A1	20021107
APPLICATION INFORMATION:	US 2001-993241		20011113

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION OF:	US 2000-711205	20001109	PENDING
CONTINUATION-IN-PART OF:	US 1999-439923	19991112	PENDING
FAMILY INFORMATION:	US 2002164758	20021107	
	US 6858206	20050222	
DOCUMENT TYPE:	Utility Patent Application - First Publication		
FILE SEGMENT:	CHEMICAL APPLICATION		

NUMBER OF CLAIMS: 28 13 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 represents the nucleotide and deduced amino acid sequences of cDNA encoding alpha-L-**iduronidase** (SEQ ID NOS:1 and 2). Nucleotides 1 through 6200 are provided. Amino acids are provided starting with the first methionine in the open reading frame.

FIG. 2 represents the results from SDS-PAGE runs of eluate obtained according to the procedures as described below. The top panel shows the SDS-PAGE results of **purified** alpha-Liduronidase (3 micrograms) and contaminants from the production/ **purification** scheme disclosed in Kakkis, et al., Protein Expr. **Purif**. 5: 225-232 (1994). In the bottom panel, SDS-PAGE results of **purified** alpha-L-**iduronidase** with contaminants from an unpublished prior production/**purification** process (U.S. patent application Ser. Nos. 09/078,209 and 09/170,977) referred to as the Carson method in Lanes 2 (7.5 microgram alpha-Liduronidase) and Lane 3 (5.0 microgram alpha-L-**iduronidase**) are compared to that of the production/**purification** process of the present invention referred to as the Galli Process (Lane 4 5 micrograms alpha-L-**iduronidase**). Lane 1 contains the molecular weight marker. FIG. 2 shows that the Galli production/ ***purification*** method of the present invention yields a highly ***purified*** alpha-L-**iduronidase** product with fewer contaminants in comparison with prior production/**purification** schemes.

FIG. 3 demonstrates the alpha-**iduronidase** production level over a 30-day period, during which time cells are switched at day 5 from a serum-containing medium to a serum-free medium. alphaIduronidase production was characterized by: (1) absence of a need for adaptation when cells are switched from serumcontaining to serum-free medium at 100200 (top and bottom panels) with an uninterrupted increase in productivity (top panel); (2) a high level of production in excess of 4 mg per liter (1000 per mL) in a protein-free medium (bottom panel); and (3) a boost in alpha-**iduronidase** production with butyrate induction events (bottom panel).

FIG. 4 demonstrates a decrease in liver volume during enzyme therapy in MPS I patients.

FIG. 5 demonstrates urinary GAG excretion during enzyme therapy.

FIG. 6 demonstrates elbow and knee extension in HAC002 during enzyme therapy.

FIG. 7 demonstrates shoulder flexion to 104 weeks in four patients with the most restriction during enzyme therapy.

FIG. 8 demonstrates improvement in sleep apnea before and after six weeks of therapy.

FIG. 9 demonstrates the improvement in apneas and hypopneas during sleep with enzyme therapy in each individual patient.

FIG. 10 demonstrates the improvement in pulmonary function tests before and after 12 and 52 weeks of enzyme therapy in one patient.

FIG. 11 demonstrates increased height growth velocity with enzyme therapy.

FIG. 12 shows the degree of contamination by Chinese Hamster Ovary Protein (CHOP) and degree of purity of alpha-Liduronidase, produced by (1) the Carson method, an unpublished prior production/**purification** process (U.S. patent application Ser. Nos. 09/078,209 and 09/170,977 and (2) the Galli method, the production/**purification** process of the present invention. Thus, FIG. 12 shows that alpha-L-**iduronidase** produced and ***purified*** by the Galli method has a higher degree of purity and lower degree of CHOP contamination in comparison to that of the Carson method.

FIG. 13 shows a comparison of alpha-L-**iduronidase** produced by the Galli method versus the Carson method. On the left side of the Figure, results from a Western Blot show that the Galli material (left side, column 2) comprise fewer contaminating protein bands (between 48 kDa and 17 kDa) in comparison with the Carson material (left side, column 3). On the right side of the Figure, results from an SDS-PAGE silver stain show the absence of a band at the 62 kDa in the Galli material (column 2) in comparison to the presence of such a band in the Carson material (column 3).

AB The present invention provides a formulation comprising a pharmaceutical composition comprising a **human recombinant** alpha -L-**iduronidase** or biologically active or muteins thereof with a

purity of greater than **99**%, or in combination with a pharmaceutically acceptable carrier. The present invention further provides methods to treat certain genetic disorders including alpha -L-**iduronidase** deficiency and mucopolysaccharidosis I (MPS 1) by administering said formulation.

L6 ANSWER 13 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN

AN	10203095 IFIPAT;IFIUDB;IFICDB
TITLE:	METHODS FOR PRODUCING AND **PURIFYING RECOMBINANT** ALPHA-L-**IDURONIDASE**; ENZYMATIC POLYPEPTIDE FOR USE IN GENETIC ENGINEERING
INVENTOR(S):	Chan; Wai-Pan, Castro Valley, CA, US Chen; Lin, San Francisco, CA, US Fitzpatrick; Paul A., Albany, CA, US Henstrand; John M., Davis, CA, US Kakkis; Emil D., Novato, CA, US Qin; Minmin, Pleasanton, CA, US Starr; Christopher M., Sonoma, CA, US Wendt; Dan J., Walnut Creek, CA, US Zecherle; Gary N., Novato, CA, US
PATENT ASSIGNEE(S):	Unassigned
PATENT ASSIGNEE PROBABLE:	Los Angeles Biomedical Res Inst at Harbor Ucla Medical Center (Probable)
AGENT:	HOWREY SIMON ARNOLD & WHITE, LLP, BOX 34, 301 RAVENSWOOD AVE., MENLO PARK, CA, 94025, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002146802	A1	20021010
APPLICATION INFORMATION:	US 2001-993038		20011113

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CONTINUATION OF:	US 2000-711202	20001109	PENDING
CONTINUATION-IN-PART OF:	US 1999-439923	19991112	PENDING
FAMILY INFORMATION:	US 2002146802	20021010	

DOCUMENT TYPE:	Utility Patent Application - First Publication
FILE SEGMENT:	CHEMICAL APPLICATION
NUMBER OF CLAIMS:	7 13 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 represents the nucleotide and deduced amino acid sequences of cDNA encoding alpha-L-**iduronidase** (SEQ ID NOS:1 and 2). Nucleotides 1 through 6200 are provided. Amino acids are provided starting with the first methionine in the open reading frame.

FIG. 2 represents the results from SDS-PAGE runs of eluate obtained according to the procedures as described below. The top panel shows the SDS-PAGE results of **purified** alpha-Liduronidase (3 micrograms) and contaminants from the production/ **purification** scheme disclosed in Kakkis, et al., Protein Expr. **Purif**. 5: 225-232 (1994). In the bottom panel, SDS-PAGE results of **purified** alpha-L-**iduronidase** with contaminants from an unpublished prior production/**purification** process (U.S. patent application Ser. Nos. 09/078,209 and 09/170,977) referred to as the Carson method in Lanes 2 (7.5 microgram alpha-Liduronidase) and Lane 3 (5.0 microgram alpha-L-**iduronidase**) are compared to that of the production/**purification** process of the present invention referred to as the Galli Process (Lane 4 5 micrograms alpha-L-**iduronidase**). Lane 1 contains the molecular weight marker. FIG. 2 shows that the Galli production/ ***purification*** method of the present invention yields a highly ***purified*** alpha-L-**iduronidase** product with fewer contaminants

in comparison with prior production/**purification** schemes.
FIG. 3 demonstrates the alpha-**iduronidase** production level over a 30-day period, during which time cells are switched at day 5 from a serum-containing medium to a serum-free medium. alphaIduronidase production was characterized by: (1) absence of a need for adaptation when cells are switched from serumcontaining to serum-free medium at 100200 (top and bottom panels) with an uninterrupted increase in productivity (top panel); (2) a high level of production in excess of 4 mg per liter (1000 per mL) in a protein-free medium (bottom panel); and (3) a boost in alpha-**iduronidase** production with butyrate induction events (bottom panel).
FIG. 4 demonstrates a decrease in liver volume during enzyme therapy in MPS I patients.
FIG. 5 demonstrates urinary GAG excretion during enzyme therapy.
FIG. 6 demonstrates elbow and knee extension in HAC002 during enzyme therapy.
FIG. 7 demonstrates shoulder flexion to 104 weeks in four patients with the most restriction during enzyme therapy.
FIG. 8 demonstrates improvement in sleep apnea before and after six weeks of therapy.
FIG. 9 demonstrates the improvement in apneas and hypopneas during sleep with enzyme therapy in each individual patient.
FIG. 10 demonstrates the improvement in pulmonary function tests before and after 12 and 52 weeks of enzyme therapy in one patient.
FIG. 11 demonstrates increased height growth velocity with enzyme therapy.
FIG. 12 shows the degree of contamination by Chinese Hamster Ovary Protein (CHOP) and degree of purity of alpha-Liduronidase, produced by (1) the Carson method, an unpublished prior production/**purification** process (U.S. patent application Ser. Nos. 09/078,209 and 09/170,977 and (2) the Galli method, the production/**purification** process of the present invention. Thus, FIG. 12 shows that alpha-L-**iduronidase** produced and ***purified*** by the Galli method has a higher degree of purity and lower degree of CHOP contamination in comparison to that of the Carson method.
FIG. 13 shows a comparison of alpha-L-**iduronidase** produced by the Galli method versus the Carson method. On the left side of the Figure, results from a Western Blot show that the Galli material (left side, column 2) comprise fewer contaminating protein bands (between 48 kDa and 17 kDa) in comparison with the Carson material (left side, column 3). On the right side of the Figure, results from an SDS-PAGE silver stain show the absence of a band at the 62 kDa in the Galli material (column 2) in comparison to the presence of such a band in the Carson material (column 3).

AB The present invention provides a **recombinant human** alpha -Liduronidase and biologically active fragments and muteins thereof with a purity greater than **99%**. The present invention further provides large-scale methods to produce and **purify** commercial grade **recombinant human** alpha -L-**iduronidase** enzyme thereof.

L6 ANSWER 14 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN

AN	10129817 IFIPAT;IFIUDB;IFICDB
TITLE:	METHODS OF **PURIFYING HUMAN** ACID ALPHA-GLUCOSIDASE; **PURIFYING** PREFERENTIAL ENZYMATIC POLPEPTIDE; OBTAIN SAMPLE, EXPOSE TO COLUMN, RECOVER ELUATE, APPLY TO COLUMN, RECOVER ENZYMATIC POLYPEPTIDE
INVENTOR(S):	Reuser; Arnold J., Rotterdam, NL Van der Ploeg; Ans T., Poortugaal, NL
PATENT ASSIGNEE(S):	Unassigned
AGENT:	NATH & ASSOCIATES, 1030 15th STREET, 6TH FLOOR, WASHINGTON, DC, 20005, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002073438	A1	20020613
APPLICATION INFORMATION:	US 2001-886477		20010622

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION-IN-PART OF:	US 2001-770253	20010129	PENDING

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	US 1995-1796P	19950802	(Provisional)
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FAMILY INFORMATION:	US 2002073438	20020613	

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
OTHER SOURCE: CA 137:17456

NUMBER OF CLAIMS: 39 24 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1: A transgene containing acid α-glucosidase cDNA. The αs1-casein exons are represented by open boxes; . alpha.-glucosidase cDNA is represented by a shaded box. The . alpha.s1-casein intron and flanking sequences are represented by a thick line. A thin line represents the IgG acceptor site. The transcription initiation site is marked (), the translation initiation site (ATG), the stopcodon (TAG) and the polyadenylation site (pA).
FIG. 2 (panels A, B, C): Three transgenes containing acid alpha.glucosidase genomic DNA. Dark shaded areas are αs1 casein sequences, open boxes represent acids alpha.-glucosidase exons, and the thin line between the open boxes represents αglucosidase introns. Other symbols are the same as in FIG. 1.
FIG. 3 (panels A, B, C): Construction of genomic transgenes. The α-glucosidase exons are represented by open boxes; the . alpha.-glucosidase introns and nontranslated sequences are indicated by thin lines. The pKUN vector sequences are represented by thick lines.
FIG. 4 (panels A and B). Detection of acid α-glucosidase in milk of transgenic mice by Western blotting.
FIG. 5. Chromatography profile of rabbit whey on a Q Sepharose FF column. A whey fraction from rabbit (line 60) milk (about 550 ml), prepared by tangential flow filtration (TFF) of the (diluted) skimmed milk, was incubated with solvent/detergent (1% Tween-80,0.3% TnBP), and loaded on a Q Sepharose FF column (Pharmacia XK-50 column, 18 cm bed height; 250 5 cm/hr flow rate). The column was washed with (7) column volumes (cv) of buffer A (20 mM sodium phosphate buffer pH 7.0), and the **human** acid a-glucosidase fraction was eluted with 3.5 cv buffer A, containing 100 mM sodium chloride. All strongly bound proteins were eluted with about 3 cv 100% buffer B (1 M NaCl, 20 mM sodium phosphate buffer pH 7.0). All column chromatography was controlled by the AKTA system of Pharmacia.
Protein was detected on-line by measuring the absorbance at 280 nm.
FIG. 6. Chromatography profile of Q Sepharose FF-**purified** ***recombinant*** **human** a-glucosidase fraction on a Phenyl HP Sepharose column.
One volume of 1 M ammonium sulphate was added to the Q Sepharose FF ***human*** acid a-glucosidase eluate (obtained with 100 mM sodium chloride, 20 mM sodium phosphate buffer pH 7.0 step; fraction F3 of FIG. 1) while stirring continuously. This sample was loaded on a Phenyl HP Sepharose column (Pharmacia XK-50 column, 14 cm bed height; 150 cm/hr flow rate) at room temperature (loaded 1-1.2 mg a-glucosidase/ml Sepharose). Before loading, the column was equilibrated in 0.5 M ammonium sulphate, 50 mM sodium phosphate buffer pH 6.0 (=buffer C). After loading the sample, the column was washed with 2 cv of buffer C to remove contaminating proteins like transferrin and serum albumin. Most **recombinant human** acid a-glucosidase was eluted from the Phenyl HP column with 4 cv buffer D(=50 mM sodium phosphate at pH 6.0 buffer). The strongest bound proteins were eluted first with water, then

with 20% ethanol.
FIG. 7. Chromatography profile of a (Phenyl HP Sepharosepurified) ***recombinant*** **human** a-glucosidase fraction on Source Phenyl 15 column. A 2 M ammonium-sulphate, 50 mM sodium phosphate buffer, pH 7.0 was added to the **human** acid aglucosidase eluate from the Phenyl HP column (fraction F4 from FIG. 6), until a final concentration of 0.85 M ammonium sulphate was reached. The solution was stirred continuously and mildly. The eluate was loaded on a Source Phenyl 15 column (Pharmacia Fineline 100 column, 15 cm bed height ;76 cm/hr flow rate) pre-equilibrated in 0.85 M ammonium sulphate, 50 mM sodium phosphate pH 7.0 buffer (=buffer E).
About 2 mg of acid a-glucosidase can be loaded per ml Source 15 Phenyl in this column. After loading the sample, **recombinant human** acid aglucosidase was eluted from the (Source 15 Phenyl) column with 10 cv of a linear gradient from 100% buffer E to 100% buffer F (buffer F =50 mM sodium phosphate buffer, pH 7.0). Careful pooling of the elution fraction is required (based on purity profiles of the column fractions on SDS-PAGE using Coomassie Brillant Blue staining) to obtain highly **purified recombinant** acid a-glucosidase. Residual bound proteins were eluted from the column, first with water, and then with 20% ethanol.
FIG. 8. SDS-PAGE analysis of various fractions during the acid aglucosidase ***purification*** procedure. Various fractions obtained during a ***recombinant*** **human** acid a-gylucosidase **purification** from rabbit milk (line 60) were diluted in non-reduced SDS sample buffer. The samples were boiled for 5 minutes and loaded on a SDS-PAGE gradient gel (4-12%, Novex).
Proteins were stained with Coomassie Brillant Blue. Lane 1: Full rabbit milk (40 ug); 2. Whey after TFF of skimmed milk (40, ug) ; 3. Acid a-glucosidase eluate fraction from the Q Sepharose FF column (30 ug); 4. Acid aglucosidase eluate fraction from the Phenyl HP column (5 ug); 5. Acid aglucosidase eluate fraction from the Source 15 Phenyl column (5 ug). The letters refer to protein bands which were identified as: a. rabbit immunoglobulins; b. unknown protein; c. **recombinant human** acid aglucosidase precursor (doublet under these SDS-PAGE conditions) ; d. rabbit transferrin, e. rabbit serum albumin; f. rabbit caseins; g. rabbit Whey Acidic Protein (WAP), possibly a dimer; h. rabbit Whey Acidic Protein (WAP), monomer; i. unknown protein, possibly a rabbit WAP variant, or a-lactalbumin; j. dimer or ***recombinant*** **human** acid a-glucosidase precursor (doublet under these SDS-PAGE conditions); k. unknown protein (rabbit transferrin, or processed **recombinant human** acid aglucosidase.
FIG. 9. HPLC size exclusion profile of **purified recombinant** ***human*** acid aglucosidase precursor. **Recombinant human** acid aglucosidase precursor was **purified** from transgenic rabbit milk by defatting milk, TFF of skimmed milk, Q FF chromatography, Phenyl HP chromatography. Source 15 Phenyl chromatography, and final filtration. The sample was prepared for the HP SEC chromatography run as described in Example 5.
FIG. 10. Binding of 1251 **human** acid a-glucosidase precursor to various metal-chelating and lectin Sepharoses. **Purified human** acid a-glucosidase precursor from rabbit line 60 was radiolabeled with 1251 as described in Example 5. Binding of the labeled enzyme to the metal-chelating Sepharoses (Fe2+, Fe3+, Cu2+, Zn2+, glycine, and control) and to the lectin Sepharoses (Concanavalin A and lentil) was done as described in Example 1. Two washing procedures were tested: either a wash with PBS, 0. 002% Tween-20 buffer, or a wash with PBS, 0.1% Tween-20,0.5 M sodium chloride buffer. The binding percentages relate to the total amount of radiolabel added to the tubes.
FIG. 11. Chromatographic elution profiles of acid a-glucosidasecontaining fractions on various HIC columns.
Purified acid a-glucosidase 110 kDa precursor or mature 76 kDa acid a-glucosidase (A and B; both 5 ug; **recombinant** from transgenic mouse milk line 2585) were analyzed on a 1 ml Butyl 4 Fast Flow Sepharose or Octyl 4 Fast Flow Sepharose HiTrap column (Pharmacia, Sweden). A transgenic (line 60;-0-) and nontransgenic (-) whey fraction (prepared by 20,000 g, 60 min

centrifugation) were also analyzed on a butyl column (both 200 ul, 25 fold diluted; C). Also a Q Fast Flow fraction (eluted at 100 ut salt from the column; see FIG. 1) of transgenic (line 60;-0-) and non-transgenic (-) whey were loaded on an ether column (both 200 ul, 25-fold diluted; Toyopearl Ether 650 M (TosoHaas) in a 2.5 ml, 5 cm bed height column; C). The results indicate a strong binding of acid a-glucosidase to the HIC columns (A and B). Most whey proteins do not bind (C). A nearly pure acid a-glucosidase was obtained after loading a Q Fast Flow eluate on an ether column (D), where most of the contaminating proteins like serum albumin and transferrin do not bind (SDS-PAGE gels not shown). The binding buffer in A, B, and C was M ammonium sulphate, 50 mM sodium phosphate pH 7.0. The binding buffer in D was 1.5 M ammonium sulphate, 50 mM sodium phosphate pH 7.0. The flow rate was 1 ml/min. Bound protein was eluted with a linear salt gradient to 50 mM sodium phosphate pH 7.0 in 30 min. All column chromatography was controlled by the AKTA system of Pharmacia. Protein was detected on-line by measuring the absorbance at 280 nm (0.2 cm flow cell). The conductivity was measured on line. mAU=milliAbsorbance units, mS/cm=milli-Siemens/cm.
FIG. 12 Chromatography profiles of transgenic and non-transgenic whey fractions on a Hydroxylapatite column. Transgenic (-) and non-transgenic (-) rabbit whey, obtained after skimming (by centrifugation) and casein removal (by TFF), were loaded on a Amberchrome column (4.6 x 150 mm) containing Macro-Prep ceramic hydroxylapatite type I (40 Ltm beads; BioRad) connected to a FPLC system of Pharmacia. Whey fractions obtained after TFF were diluted 5-fold in buffer A (10 mM NaPi pH 6.8), and 0.2 ml was loaded on the column pre-equilibrated in buffer A. The flow rate was 2 ml/min. After loading, bound protein was eluted with a gradient to 500 mM NaPj pH 6.8 in 10 column volumes. Protein was detected by measuring the absorbance at 280 rn (flow cell is 2 mm).
FIG. 13. SDS-PAGE analysis of whey fractions from the hydroxylapatite column. Transgenic and non-transgenic rabbit whey were loaded on the Macro-Prep ceramic hydroxylapatite type) column as described in FIG. 12.
Flow through and eluate fractions were obtained, which were analyzed on SDS-PAGE (for details of the gels see FIG. 8). A. silver stained SDS PAGE of transgenic whey run on hydroxylapatite; B. silver stained SDS PAGE of non-transgenic whey. Up to 6 g protein was loaded.

DESCRIPTION OF FIGURES:

FIGS. 14 to 19 are chromatograms of hydroxylapatite chromatography separations of transgenic whey samples in which the samples were loaded on to the column at sodium phosphate buffer (NaPi) concentrations of 5,10, 20,30,40 or 50 mM respectively. The pH of the buffer was 7.0. The chromatograms show the gradient of sodium phosphate eluting buffer to 400 mM, the AZSO and the pH of the eluate and the fractions collected.
FIGS. 20 to 23 are chromatograms of hydroxylapatite chromatography separations as in FIGS. 14 to 19 above except that the pH of the sample was varied whilst the NaPi buffer concentration was retained at 5 mM. The pH of the samples fractionated were pH 6.0,7.0 and 7.5respectively.
FIG. 24 is a chromatogram of an industrial (pilot) scale separation of transgenic milk whey on Q Sepharose FF.
FIG. 25 is a chromatogram of hydroxylapatite column chromatography of 0.1 M eluate from the Q Sepharose FF column.
FIG. 26 is a silver stained SDS-PAGE gel of flow through fractions from a series of hydroxylapatite chromatography separations of 0.1 M eluates of Q Sepharose FF. !

AB The invention provides methods of **purifying** lysosomal proteins, pharmaceutical compositions for use in enzyme replacement therapy, and methods of treating Pompe's disease using **purified** **human** acid alpha glucosidase.

L6 ANSWER 15 OF 16 IFIPAT COPYRIGHT 2006 IFI on STN
AN 03725200 IFIPAT;IFIUDB;IFICDB
TITLE: **RECOMBINANT** ALPHA-L-**IDURONIDASE**, METHODS FOR PRODUCING AND **PURIFYING** THE SAME AND METHODS FOR TREATING DISEASES CAUSED BY DEFICIENCIES THEREOF; ISOLATED POLYPEPTIDE

INVENTOR(S): Kakkis; Emil D., Long Beach, CA
Tanamachi; Becky, Signal Hill, CA
PATENT ASSIGNEE(S): Harbor-UCLA Research and Education Institute, Torrance, CA
PRIMARY EXAMINER: Achutamurthy, Ponnathapu
ASSISTANT EXAMINER: Rao, Manjunath N
AGENT: Chiang, Robin C.Howrey Simon Arnold & White, LLP
Halluin, Albert P.

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APPLICATION INFORMATION:	US 1999-439923		19991112
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CORRECTION DATE: 28 Jan 2003
FILE SEGMENT: CHEMICAL
GRANTED

MICROFILM REEL NO: 010728 FRAME NO: 0428
NUMBER OF CLAIMS: 7
GRAPHICS INFORMATION: 15 Drawing Sheet(s), 16 Figure(s).
AB The present invention provides a **recombinant** alpha -Liduronidase and biologically active fragments and mutants thereof, methods to produce and **purify** this enzyme as well as methods to treat certain genetic disorders including- alpha -Liduronidase deficiency and mucopolysaccharidosis I (MPS 1).

L6 ANSWER 16 OF 16 PROMT COPYRIGHT 2006 Gale Group on STN

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